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Recent measurements of electron-transfer (ET) rates in $Ru(NH_3)_5$ His myoglobin derivatives have shown the need for improved theories for treating the "path" of long-range ET. We have investigated these systems theoretically using a combined artificial intelligence (AI)–superexchange method. As in our previous articles, this model first employs an AI search technique that yields the important amino acid residues for the mediation of electrons between the donor and the acceptor. A quantum mechanical method is then used to diagonalize the orbitals of the selected protein subset and to calculate the electronic coupling. Encouraging agreement with experimental data is found, no adjustable parameters having been introduced. The results yield the relevant amino acid paths.

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Electron-Transfer Reactions in Proteins: Electronic Coupling in Myoglobin

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Recent measurements of electron-transfer (ET) rates in $Ru(NH_3)_5$ His myoglobin derivatives have shown the need for improved theories for treating the "path" of long-range ET. We have investigated these systems theoretically using a combined artificial intelligence (AI)–superexchange method. As in our previous articles, this model first employs an AI search technique that yields the important amino acid residues for the mediation of electrons between the donor and the acceptor. A quantum mechanical method is then used to diagonalize the orbitals of the selected protein subset and to calculate the electronic coupling. Encouraging agreement with experimental data is found, no adjustable parameters having been introduced. The results yield the relevant amino acid paths.

Introduction

Long-range electron transfer (ET) is central to many biological processes such as respiration and photosynthesis. Electron-transfer reactions in biological systems such as proteins have therefore been the subject of intense experimental and theoretical studies.[1-3] In theories of electron-transfer reactions, factors which influence the rate of these reactions include the driving force ($-\Delta G^\circ$), a nuclear reorganization term (λ), the separation distance (R), and the nature of the medium separating the electron donor and the acceptor.[4] In order to probe unambiguously the effect of the intervening medium on the rate of electron-transfer reactions in proteins, there have been several experimental investigations of intramolecular electron-transfer reactions in native and modified proteins.[5-7] In this paper, we present results of theoretical studies on the distance and medium dependences of the rates of electron-transfer reactions in proteins, particularly the ruthenium-modified myoglobin derivatives studied experimentally by Gray and co-workers.[8]

For nonadiabatic electron-transfer reactions, such as the long-range ET reactions in proteins, the rate constant for transfer of an electron from a donor to an acceptor can be expressed in terms of a Golden Rule type expression, namely, as the product of the square of an electronic coupling matrix element (H_{DA}) and a nuclear Franck–Condon factor (FC):[3]

$$k_{ET} = \frac{2\pi}{\hbar}|H_{DA}|^2(FC) \qquad (1)$$

The Franck–Condon factor (FC) can be estimated quantum mechanically, semiclassically, or classically. Various expressions for FC are given, for example, in a recent review.[9] In the classical limit, the Franck–Condon factor is given by

$$FC = \frac{1}{(4\pi\lambda RT)^{1/2}} \exp\left(-\frac{(\Delta G^\circ + \lambda)^2}{4\lambda RT}\right) \qquad (2)$$

where ΔG° is the standard free energy for the electron-transfer reaction at a fixed donor–acceptor separation distance R and λ is a reorganizational term which contains both solvent (protein) and vibrational contributions.

The electronic matrix element, H_{DA}, describes the coupling of the orbitals of the donor (D) with the acceptor (A). Simple square barrier tunneling models[10] have suggested an exponential decay of H_{DA} with the distance R separating the donor and the acceptor:

$$H_{DA} = H^0_{DA} \exp(-\beta(R - R^0)/2) \qquad (3)$$

where H^0_{DA} is the matrix element at van der Waals contact, R^0 ($R^0 \sim 3$ Å), and β is the distance decay factor. Such an exponential dependence has been experimentally and theoretically verified for several synthetic D–A systems,[11-18] where β has been found to be between 0.8 and 1.2 Å^{-1}. A recent analysis of ET rates in several biological systems, most particularly the bacterial photosynthetic reaction centers, by Dutton and co-workers has provided an overall support for the exponential decay model for H_{DA} in biological ET reactions as well.[19] However, in electron-transfer systems which are not structurally homogenous, such as proteins, the electronic structure of the protein medium coupling D and A, in addition to the separation distance, is expected to affect H_{DA}.

It has been shown[20] that the electronic coupling in derivatives of cytochrome c does not scale uniformly with distance. The electronic coupling in these proteins is, on the other hand, satisfactorily explained by simple pathway models[21] as well as by more sophisticated models which explicitly take into account the electronic structure of the protein medium.[22,23] The model that we have previously introduced[22,24] to treat electronic interactions in proteins combines an artificial intelligence (AI) approach with a quantum mechanical formulation of superexchange, without the introduction of any adjustable parameters. The relative values of electronic coupling elements obtained with this model were in good agreement with experimental results for the cytochrome c derivatives.

Recently, Gray and co-workers have experimentally studied ET in Ru-modified myoglobin derivatives.[8] As noted by these authors, a simple pathway analysis does not adequately describe the electronic coupling in these systems. Nor did a multiple pathway model which neglected interferences between pathways. Presently, we investigate the electronic interactions in these myoglobin derivatives using our AI–superexchange method.

Theory

For donor–bridge–acceptor (D–B–A) electron-transfer systems, where the distance between D and A spans 10–20 Å, the direct

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electronic coupling between D and A is negligible and the protein (the "bridge") provides the electronic coupling which enables the electron-transfer reaction. Second-order perturbation theory can be used to determine this bridge-mediated electronic coupling.[25,26] The electronic properties of the bridge, namely, the molecular orbitals and their eigenvalues, are calculated separately, and the interaction of the donor/acceptor with the bridge is treated as a perturbation. In a one-electron description of the D–B–A system, this "superexchange" mechanism can be formulated as

$$H_{DA} = \sum_{\alpha} \frac{T_{\alpha}^{D} T_{\alpha}^{A}}{\Delta E_{\alpha}} \qquad (4)$$

where T_{α}^{D} (T_{α}^{A}) represents the interaction of the donor (acceptor) orbital with the bridge orbitals and ΔE_{α} is the energy of the bridge orbitals relative to the energy of the donor orbital. The above formulation includes hole transfer as well as electron transfer, since the summation over α runs over both occupied and unoccupied orbitals of the bridge. Expressions for T_{α}^{D}, T_{α}^{A}, and ΔE_{α} can be found in a previous article (see eq 8 in ref 22).

A direct implementation of such a formulation for the case of long-range ET in proteins requires determining the electronic wave functions of the protein, which serves as the bridge in such reactions. This step necessitates diagonalization of a large matrix (of the order of 6200 × 6200 for myoglobin) and is a computationally formidable task and less illuminating, we believe, than an alternative procedure. We have previously shown[22,24] that, to adequately describe the electronic coupling provided by the protein medium, it is not necessary to include all the amino acid residues of the protein but only a suitably chosen subset of the protein. This subset of the protein is selected by use of an artificial intelligence search algorithm. We briefly describe below the way the search operates but refer the reader to earlier papers[22,24] for a more complete account.

The search starts from the donor site D in the protein and attempts to locate the acceptor site A by traveling via the intermediate atoms I of the amino acid residues of the protein molecule. These intermediate atoms or "nodes" are chosen on the basis of an evaluation function, EF, which is a measure of the coupling of the node I to the donor (V_{DI}) as well as an estimate of the coupling from I to the acceptor (T_{IA}):

$$(EF)_I = V_{DI} T_{IA} / \Delta E \qquad (5)$$

where ΔE is a suitably chosen energy difference. The couplings V_{DI} and T_{IA} can be estimated as explained in section II of ref 22. It is important to emphasize here that the evaluation function is used only for the purposes of the AI search, and the actual electronic coupling matrix element H_{DA} is calculated using eq 4.

Using the evaluation function as a heuristic to guide the search, the AI search proceeds from the donor atom through the protein molecule to the acceptor atom. Several paths, within a preset threshold value for the net electronic coupling, are determined. The search is performed in both directions, i.e., starting from the donor and reaching the acceptor, as well as starting from the acceptor and reaching the donor. The search thus maps out a number of intermediate protein atoms which enable it to proceed from one redox center to the other. The amino acid residues to which these atoms belong are then considered as the relevant amino acids for mediating the electron transfer in the protein. The search does not necessarily proceed along the backbone of the protein but instead chooses the paths that couple in the most optimal way the donor and the acceptor atoms, based on both the overlap of the intervening protein atomic orbitals as well as their energies. Thus, some of the selected amino acids will be bonded to others not selected by the search. These "dangling" bonds are completed by addition of H atoms. The selected amino acids constitute the subset of the protein that will be used as the bridge in the calculation of H_{DA}.

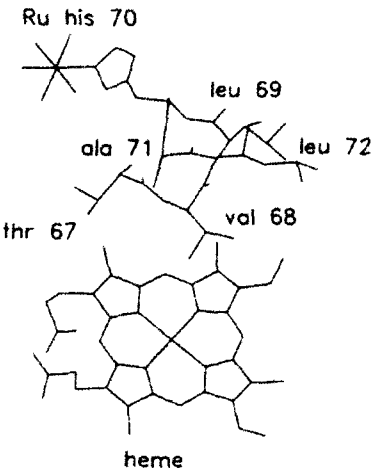

Figure 1. Amino acid residues determined by the AI search for the His 70 derivative of myoglobin.

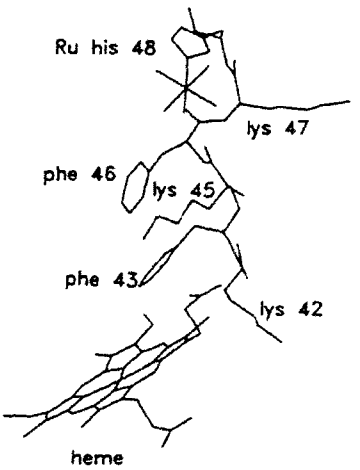

Figure 2. Amino acid residues determined by the AI search for the His 48 derivative of myoglobin.

Results and Discussion

The proteins considered here are the ruthenium-modified myoglobin derivatives studied experimentally by Gray and coworkers. Three mutant human myoglobins were modified by replacing the heme by Zn mesoporphyrin (ZnP) and coordinating a pentaammineruthenium complex to a surface histidine residue (Ru His X Mb, where X = 70, 48, and 83). In the resulting singly ruthenated myoglobin derivatives, ET rates from ^{3}ZnP* to Ru^{3+} were measured using transient absorption spectroscopy.[8] The driving force for the reaction is 0.82 eV, and the reorganization energy, λ, has been estimated to be 1.3 eV from a study of the driving force dependence of ET rates in Ru His 48 Mb.[26] Activationless ET rates, k_{max}, were extracted from the observed ET rates, assuming λ = 1.3 eV for all the three derivatives.

In the calculational procedure, the donor is the Zn porphyrin group and the acceptor is the ruthenated histidine group. The coordinates of the Ru-modified myoglobins were obtained from the refined protein coordinates[27] by performing a search of the ruthenated histidine conformations. The two side-chain dihedral angles, C_{α}–C_{β} and C_{β}–C_{γ}, were each rotated by 1° from 0° to 360° to find the most stable conformer.[28] The resulting structure files were used in the AI search.

For each of the three derivatives, His 70 Mb, His 48 Mb, and His 83 Mb, the search was allowed to select several paths, as described earlier. A preset threshold value for the net electronic coupling of any path was used to exclude particularly unimportant paths.[29] Figures 1–3 show the amino acid residues that were selected by the search for each of the three myoglobin derivatives.

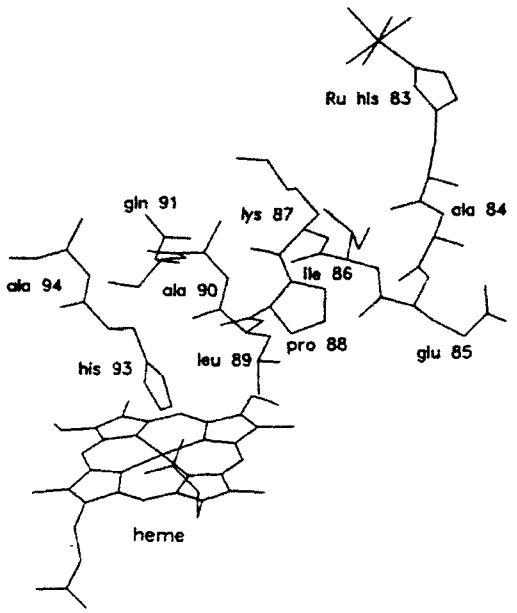

Figure 3. Amino acid residues determined by the AI search for the His 83 derivative of myoglobin.

TABLE I: Calculated Electronic Couplings and Electron-Transfer Rates

			$(k_{max,i}/k_{max,2})^{1/2}$	
i (deriv)	R (Å)	H_{DA} (cm^{-1}) calc	calc	expt[a]
1 (His 70)	11.1	5.9×10^{-1}	26	15
2 (His 48)	13.8	2.0×10^{-2}	1	1
3 (His 83)	17.6	7.0×10^{-4}	0.03	0.07

[a] Reference 8.

Only 5–10 of the 153 possible amino acid residues are determined by the AI search as important in these protein-mediated ET reactions.

A quantum mechanical calculation of the electronic coupling matrix element was then performed using, as the bridge, the amino acid residues picked by the AI search. As in earlier papers,[22,24] extended Hückel theory[30] was used to calculate H_{DA}. These electronic couplings are given in Table I. From these electronic couplings, we can then estimate k_{max}, the maximum rate constant for ET in these three derivatives as follows: The maximum rate constant for nonadiabatic ET reactions occurs when the driving force equals the reorganization energy, i.e., when the exponential factor in eq 2 is unity. The maximum or activationless rate constant is then given by eq 6 (using a classical approximation):

$$k_{max} = \frac{2\pi}{\hbar}|H_{DA}|^2 \frac{1}{(4\pi\lambda RT)^{1/2}} \qquad (6)$$

The calculated k_{max} values are (in s^{-1}) 8.0×10^7, 1.2×10^5, and 1.1×10^2 for the His 70 Mb, His 48 Mb, and His 83 Mb derivatives, respectively. The maximum rates extracted from the experimentally observed rates[8] are 7.2×10^7, 3.2×10^5, and 1.8×10^3 for these same derivatives. The good agreement between these absolute values should be regarded as fortuitous, and it is preferable to consider, instead, relatives values when comparing calculated and experimental results, as in Table I: Results from extended Hückel calculations are more reliable for relative values of H_{DA} and hence k_{max} than for absolute values.[31] Further, the "absolute" values of k_{max} extracted from experimental data are also dependent on the model chosen to correct for the nuclear factor and might change somewhat with a different analysis of the experimental data. (In ref 8 and here, the simplest model, namely, the exponential expression in eq 2, has been chosen, and fixed λ and ΔG° have been assumed for all the three ET reactions in obtaining both the calculated and experimental k_{max} values.

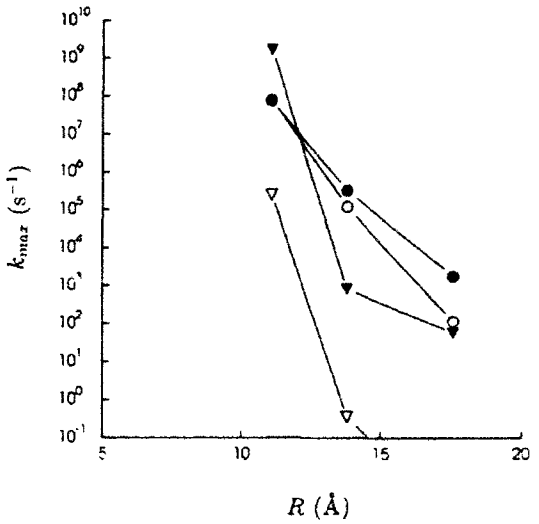

Figure 4. Variation of calculated and experimental k_{max} with distance in the Ru-modified myoglobin derivatives. The filled circles refer to the experimental values, the hollow circles refer to the present calculations, the filled triangles refer to the multiple pathway results of ref 8, and the unfilled triangles refer to their single pathway results. The experimental and calculated k_{max} for the His 70 Mb derivative (R = 11.1 Å), 7.2×10^7 and 8.0×10^7 s^{-1}, respectively, are too close to be distinguished in this plot. The k_{max} obtained by the single pathway analysis for His 83 Mb (R = 17.6 Å) is 7.5×10^{-4} s^{-1}, which is off this graph. As noted in the text, it is more important to consider the variation of these k_{max} values rather than their absolute values.

The resulting "extrapolation" from k_{ET} to yield k_{max} is not a large one—a factor of about 5.) Hence, it is preferable to compare the relative values of k_{max} for these various derivatives, and, therefore, *in Table I we give the ratios of k_{max} with respect to the His 48* Mb derivative. We have also plotted the variation of both the calculated and experimental k_{max} values with distance in Figure 4. (We have chosen to use the porphyrin edge–Ru distances as being the most appropriate for the plot, since the ET reactions studied are ^{3}ZnP* → Ru^{3+} and in ^{3}ZnP* the extra electron resides on the porphyrin.)

The calculated variation of k_{max} is similar to the experimentally found variation, both of them showing an approximately exponential dependence with distance. However, the calculated values exhibit a steeper falloff with distance. In order to test the convergence of the search procedure, more tolerant cutoff values were used in the AI search enabling the selection of more amino acid residues per derivative. The additional amino acids selected were His 64 for the His 70 Mb derivative; Asp 44, Leu 49, Asp 60, His 64, and His 93 for the His 48 Mb derivative; and His 82, Ser 92, and Phe 138 for the His 83 Mb derivative. The values of H_{DA} obtained with this more comprehensive calculation were 6.6×10^{-1}, 3.6×10^{-2}, and 4.2×10^{-4} cm^{-1} and the ratios of the square root of k_{max} were 18, 1, and 0.01 for His 70 Mb, His 48 Mb, and His 83 Mb, respectively (normalized with respect to the His 48 Mb derivative). Comparing with the calculated values of H_{DA} in Table I, it is seen that there is no dramatic change in H_{DA} due to the addition of more amino acid residues.

An uncertainty in the present calculation is the energy difference in the denominator of eq 4, which depends on the calculated value of the energy of the donor orbital at the transition state. In previous studies,[22,32] we have made use of experimental charge-transfer spectra from the donor species to the bridge to determine this energy difference. Presently, this energy was set to be the energy of the LUMO of the ZnP group, since, as noted earlier, the reaction studied experimentally involves transfer of an electron from the excited state, ZnP*. This LUMO is delocalized over the porphyrin ring, and, hence, extended Hückel theory was thought to be adequate enough to calculate this energy relative to the energy of the bridge orbitals, in contrast to the case where the donor orbital is localized on a metal center. This energy,

without further correction, was taken to be the energy of the donor orbital, which is equal to that of the acceptor orbital in the transition state. The present work also neglects any effects due to conformational fluctuation of the protein coordinates on the electronic coupling.

For comparison, the values of k_{max} from the Beratan–Onuchic pathway analyses of these same derivatives from ref 8 are also plotted in Figure 4. A B–O pathway is a single chain of atoms connecting the donor and the acceptor and the electronic coupling provided by a pathway is estimated by the number of links where each bond, hydrogen bond and through-space link contributes a parametrized decay factor. The two B–O pathway analyses in ref 8 differ, in that one of them uses a single pathway to estimate the electronic coupling while the other uses many such pathways and sums the electronic couplings of the individual pathways to yield the total coupling, neglecting interferences between pathways. Again, it is important to note that in the pathway analyses of ref 8 only the variation of k_{max}'s can be compared, since those analyses do not include the interaction between the donor (acceptor) with the first (last) bond of the pathway, which can cause a shift in the absolute values. As was pointed out by the authors of ref 8, while the inclusion of multiple pathways improves the agreement, neither of the two pathway analyses results in satisfactory agreement with the experimental data. Our procedure of estimating the electronic coupling automatically incorporates all possible amino acid paths within the subset of protein selected and further includes all possible interferences between these paths. It has been shown that inclusion of both the interferences between multiple paths and the non-nearest-neighbor interactions are important for describing electronic coupling in model complexes and proteins.[23,33]

It is of interest to compare the amino acid residues selected by the present AI search with the (atoms of the) amino acid residues involved in the pioneering pathway analyses of Beratan and coworkers.[21] At present, only for the His 48 derivative have the amino acid residues from the pathway approach been identified. For this derivative, the pathway analysis yields 211 paths. All of the amino acid residues involved in these 211 paths, except one, are selected by the AI search (see Figure 2). The additional amino acid present in the pathway treatment is Asp 44. We find that our AI search proceeds from Phe 43 directly to Phe 46 or Lys 45 via through-space connections rather than involving Asp 44. It involves, only, in effect, 2 or 3 amino acid chain paths instead of 211 individual atom-by-atom paths. When Asp 44 was added to the small group of amino acids depicted for the His 48 derivative in Figure 2, H_{DA} changed only by a small amount from the value of 0.020 cm^{-1} in Table I, namely to 0.023 cm. Asp 44 did contribute, together with four other amino acids, to the larger set mentioned earlier for the His 48 derivative.

Conclusions

In the present paper, we have studied the intramolecular electron-transfer reactions in the ruthenium-modified myoglobin derivatives, where recent rate measurements have shown the need for theories more sophisticated than the existing pathway analyses and homogenous barrier models. We have investigated these systems theoretically using a combined artificial intelligence search and superexchange method. This model first employs an AI search technique that yields the important amino acids for the electron-transfer reaction and then a quantum mechanical method is used to diagonalize the orbitals of the selected protein subset and to calculate the electronic matrix element. Satisfactory agreement with experimental data is found. A similar analysis has been previously implemented for the ruthenium-modified cytochrome *c* proteins and was shown to give results consistent with experimental data.[22] The present approach can thus describe the electronic coupling in both cytochrome *c* and myoglobin derivatives without introducing any adjustable parameters and offers a Hamiltonian-based method, which incorporates any number of amino acid paths and their potential interactions to treat electron transfer in proteins. We plan next to investigate ET reactions of particular biological relevance as in protein–protein complexes.

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(28) For each value of the C_α–C_β angle, the C_β–C_γ angle was rotated by 1° from 0° to 360°. Thus a total of 360 × 360 conformations were searched.
(29) The cutoff values used were 10^{-10} for His 70 Mb, 10^{-14} for His 48 Mb, and 10^{-16} for His 83 Mb. It is necessary to use lower values for the derivatives with longer $D - A$ separation in order to accommodate the lower coupling they are expected to have.
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